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(71) Applicant: LJL BIOSYSTEMS [US/US]; 404 Tasman Drive, Sunnyvale, CA 94089 (US).

(72) Inventor: LEYTES, Lev, J.; 443 Tennyson Avenue, Palo Alto, CA 94301 (US).

(74) Agent: FLOYD, Mark, B.; 426 Lowell Avenue, Palo Alto, CA 94301 (US).

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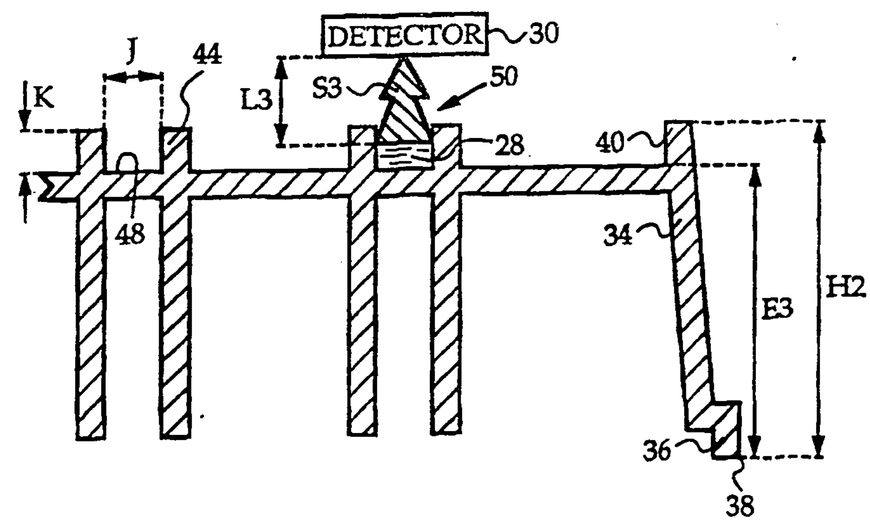

(57) Abstract

A microplate for holding mixtures (28) of reagents and samples to be assayed by emission of light signals from the mixtures to an optical detector (30) positioned above the microplate. The microplate includes a frame (34) having a base (36) and a top portion (40) extending from the base. The base has a bottom edge (38) and the top portion (40) extends to a height (H2) above the bottom edge (38). The height (H2) is preferably 14.35 mm so that the microplate is compatible with standard tray handling equipment. The microplate also includes a plurality of wells (50) disposed in the frame (34) for holding the mixtures (28). Each well has sidewalls (44) and a well bottom (48). Each well bottom is elevated a height (E3) above the bottom edge (38) of the base (36). The height (E3) is in a range of one half to nine tenths of the height (H2). The elevation of each well bottom reduces the length of the signal path (S3) from each mixture (28) to the optical detector (30) and allows a minimum quantity of reagents to be used in each well.

MICROPLATE HAVING WELLS WITH ELEVATED BOTTOMS

RELATED APPLICATION INFORMATION

This application claims priority from U.S. application Ser. No. 08/840,553 filed April 14,1997.

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to multi-well bioassay trays, which are commonly referred to as microplates, and in particular to a microplate having wells with elevated bottoms.

DESCRIPTION OF PRIOR ART

A microplate is often used to hold mixtures of reagents and samples to be assayed by techniques which are dependent upon the emission of light signals from the mixtures. The light signals are detected by an optical detector positioned above the microplate. The light signals must be sufficiently strong to be accurately detected. It is also desirable to use a minimum quantity of reagents to reduce the cost of the assay.

The current standard for microplates is a 96 well thermoplastic tray. The wells are conventionally arranged in an 8x12 array on 9 mm centers. FIG. 1 shows a cross sectional view of a portion of a conventional microplate **100**. Microplate **100** includes a frame **112** having a base **116** and a top portion **120** extending from base **116**. Base **116** has a bottom edge **118**. Top portion **120** extends to a maximum height **H1** above bottom edge **118**. The current standard for height **H1** is 14.35 mm.

Each well **114** in microplate **100** has sidewalls **122** and a bottom wall **124** attached to sidewalls **122**. The top surface of bottom wall **124** forms a well bottom **126**. Bottom wall **124** is typically 1.0 mm thick and elevated a height **E1** above bottom edge **118** to prevent scratching of bottom wall **124** when microplate **100** is

placed upon a work surface or stacked on top of another microplate. The current standard for height **E1** is 1.0 mm. Well **114** typically has a volume capacity of 300 to 400 microliters for holding a mixture **128** of sample and reagent liquids.

A chemical reaction between the sample and reagent liquids causes the generation of a light signal. The light signal travels out of well **114** along a signal path **S1** to an optical detector **130**. Signal path **S1** has a length **L1** which is dependent upon the depth of well **114**, the depth of mixture **128**, and the placement of optical detector **130** above well **114**. The depth of well **114** is typically 12.35 mm and optical detector **130** is generally placed 2.0 to 5.0 mm above well **114** to prevent contamination of the detector. The depth of mixture **128** is dependent upon the volume of well **114** occupied by mixture **128**.

Many current assay techniques require a mixture having a volume of only 10 to 45 microliters, so that the depth of mixture **128** in well **114** is typically 0.4 to 2.0 mm. Thus, length **L1** of signal path **S1** is typically 12.35 to 17.0 mm. The light signals generated by a 10 to 45 microliter mixture lack sufficient strength to travel this path length and be accurately detected by detector **130**. Consequently, a significant part of each signal is lost, reducing the sensitivity of the assay. The signal strength may be increased by using a much larger volume of reagents, but this practice adds significantly to the cost of the assay.

Recently, a few manufacturers have introduced 384 well plates in place of standard 96 well plates. The 384 well plates have the same frame dimensions as the 96 well plates, but the diameter of each well is reduced. The center to center distance between adjacent wells is also reduced, typically to 4.5 mm. The 384 well plates have the same disadvantage of creating a prohibitively long signal path between each sample mixture and an optical detector placed above the mixture. The problem of accurate signal detection is further compounded with 384 well plates because their reduced well diameter creates an even narrower signal path from the bottom of each well to the optical

detector. Thus, a significant part of each signal is lost, reducing the sensitivity of the assay.

OBJECTS AND ADVANTAGES OF THE INVENTION

In view of the above, it is an object of the present invention to provide a microplate which reduces the volume of reagent required in each well. It is another object of the invention to provide a microplate which reduces the length of the signal path from the bottom of each well to an optical detector positioned above the microplate, thereby increasing the sensitivity of an assay.

These and other objects and advantages will become more apparent after consideration of the ensuing description and the accompanying drawings.

SUMMARY

The invention presents a microplate for holding mixtures of reagents and samples to be assayed by emission of light signals from the mixtures to an optical detector positioned above the microplate. The microplate includes a frame having a base and a top portion extending from the base. The base has a bottom edge and the top portion extends to a first height above the bottom edge. The first height is in a range of 12.0 to 16.0 mm, with a preferred value of 14.35 mm so that the microplate is compatible with standard plate handling equipment.

The microplate also includes a plurality of wells disposed in the frame for holding the mixtures. In the preferred embodiment, the wells are disposed in an eight-by-twelve rectangular array such that the center of each well is spaced 9.0 mm from the center of each horizontally adjacent well and spaced 9.0 mm from the center of each vertically adjacent well. This standard 9.0 mm center to center spacing ensures that the microplate is compatible with conventional liquid transfer equipment. In a second embodiment, the wells are disposed in an sixteen-by-twenty-four rectangular array such that the center of each well is spaced 4.5 mm from the center of each horizontally adjacent well and spaced 4.5 mm from the center of each vertically adjacent well.

Each well has sidewalls and a well bottom. The well bottom is elevated a second height above the bottom edge of the base. The second height is in a range of one half to nine tenths of the first height. The elevation of each well bottom reduces the length of the signal path from each mixture to the optical detector and allows a minimum quantity of reagents to be used in each well.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a cross sectional view of a portion of a conventional microplate.

FIG. 2 is a top plan view of a microplate according to a preferred embodiment of the invention.

FIG. 3 is a cross sectional view of the microplate of FIG. 2 taken along the line A - A' in FIG. 2.

FIG. 4 is a cross sectional view of a well disposed in the microplate of FIG. 2.

FIG. 5 is a cross sectional view of other wells according to the invention.

FIG. 6 is a cross sectional view of wells having V-shaped bottoms according to the invention.

FIG. 7 is a cross sectional view of wells having U-shaped bottoms according to the invention.

FIG. 8 is a cross sectional view of wells having individual filter plug bottoms according to the invention.

FIG. 9 is a cross sectional view of wells having filter bottoms formed from a sheet of filter material.

FIG. 10 is a top plan view of a microplate having well strips according to the invention.

FIG. 11 is a cross sectional view of the microplate of FIG. 10.

FIG. 12 is a top plan view of another microplate according to the invention.

DETAILED DESCRIPTION

A preferred embodiment of the invention is shown in FIGS. 2-4. FIG. 2 shows a top plan view of a microplate **32** having a frame **34** and ninety-six wells **42**. Wells **42** are disposed in frame **34** in an eight-by-twelve rectangular array such that the center of each

well is spaced a distance **C** from the center of each horizontally adjacent well. The center of each well is also spaced a distance **C** from the center of each vertically adjacent well. Distance **C** is preferably 9.0 mm so that microplate **32** is compatible with standard liquid transfer equipment.

FIG. 3 shows a cross section of the microplate taken along the line A - A' in FIG. 2. Frame **34** has a base **36** and a top portion **40** extending from base **36**. Base **36** has a bottom edge **38**. Top portion **40** extends to a first height **H2** above bottom edge **38**. Height **H2** is preferably in the range of 12.0 to 16.0 mm, with a preferred value of 14.35 mm. The height of 14.35 mm is presently preferred so that the microplate is compatible with standard plate handling equipment.

Each well **42** has sidewalls **44** extending vertically to a second height above bottom edge **38**. The second height is preferably equal to height **H2**. Each well also has a bottom wall **46** whose upper surface forms a well bottom **48**. In the preferred embodiment, each well bottom **48** is flat. Bottom wall **46** is attached to sidewalls **44** such that each well bottom is elevated a third height **E2** above bottom edge **38**. Height **E2** is one half to nine tenths of height **H2**. Thus, in the preferred embodiment, height **E2** is in the range of 6.0 to 14.4 mm with a preferred range of 7.2 to 12.9 mm when height **H2** is equal to 14.35 mm.

FIG. 4 shows a detailed cross sectional view of the structure of each well. Each well is designed to hold a mixture **28** of reagent and sample liquids to be assayed by the emission of light signals from mixture **28** to an optical detector **30**. Mixture **28** has a depth **I** which is dependent upon a volume **V** of mixture **28**. Detector **30** is positioned a height **G** above the well. Height **G** is selected such that detector **30** is located as close as possible to the well without becoming contaminated by samples and reagents. Height **G** is typically 2.0 to 5.0 mm with a preferred value of 3.0 mm.

In the preferred embodiment, each well has a diameter **D** in the range of 6.0 to 8.0 mm with a preferred value of 7.0 mm. Also in

the preferred embodiment, each well has a depth **F** in the range of 1.5 to 7.1 mm with a preferred value of 3.0 mm. The diameter and depth of each well is preferably selected such that the well has a volume capacity of 100 to 150 microliters. Microplate **32** is easily fabricated using conventional injection molding techniques and is preferably a single molded plastic part.

The operation of the preferred embodiment is illustrated in FIG. 4. A chemical reaction between the sample and reagent liquids causes the generation of a light signal. The light signal travels out of the well along a signal path **S2** to optical detector **30**. Signal path **S2** has a length **L2**, where **L2** = **F** + **G** - **I**. Depth **I** is calculated by the following equation:

$$I = \frac{V}{\pi(D / 2)^2}$$

For example, when diameter **D** equals 7.0 mm, depth **I** ranges from 0.25 to 1.2 mm as volume **V** ranges from 10 to 45 microliters. Thus, when using the preferred values of depth **F** and height **G** given above, length **L2** of signal path **S2** ranges from 4.80 to 5.75 mm as volume **V** ranges from 10 to 45 microliters. The light signal generated by a 10 to 45 microliter mixture of sample and reagents is capable of traveling this path length with sufficient strength to be accurately detected by detector **30**.

Thus, microplate **32** provides a significant improvement over conventional microplates by allowing sensitive assays to be performed with a minimum quantity of reagents. The microplate improves the accuracy of the assay while simultaneously reducing costs. A further advantage of microplate **32** is that it achieves both of these goals while maintaining the frame dimensions and well center spacing necessary to be compatible with standard plate handling and liquid transfer equipment.

FIG. 5 shows a second embodiment of the invention in which the microplate includes a plurality of wells **50** in place of wells **42**. Wells **50** are preferably disposed in frame **34** in the same manner as wells **42**, e.g. 96 wells arranged in an 8x12 array on 9.0 mm centers. However, wells **50** differ from wells **42** in that wells **50**

have a smaller volume capacity for smaller volume applications. Each well **50** has a diameter **J** in the range of 2.0 to 5.0 mm and a depth **K** in the range of 1.5 to 4.0 mm. The diameter and depth of each well **50** is preferably selected such that each well has a volume capacity of 10 to 50 microliters.

In operation, light signals from mixture **28** travel to detector **30** along a signal path **S3**. Signal path **S3** is narrower than signal path **S2** due to the smaller diameter of wells **50** relative to wells **42**. To compensate for the narrower signal path, each well bottom **48** is elevated to a height **E3** above bottom edge **38**. Height **E3** is preferably seven tenths to nine tenths of height **H2**, e.g. 10.0 to 12.9 mm when height **H2** is equal to 14.35 mm. This elevation of each well bottom **48** ensures that optical detector **30** receives an adequate signal from mixture **28**. Other than the differences described, the operation and advantages of the second embodiment are the same as the preferred embodiment described above.

FIGS. 6 - 7 illustrate alternative embodiments of the microplate in which the well bottoms have different shapes. FIG. 6 shows wells **52** having V-shaped well bottoms. Wells **52** are preferably disposed in frame **34** in the same manner as the wells of the preferred embodiment, e.g. 96 wells arranged in an 8x12 array on 9.0 mm centers. However, wells **52** differ from the wells of the preferred embodiment in that wells **52** have bottom walls **54** whose upper surfaces form V-shaped well bottoms **56**. Bottom walls **54** are positioned such that the lowest point of each well bottom **56** is elevated height **E2** above edge **38**.

FIG. 7 shows wells **58** having U-shaped well bottoms. Wells **58** are preferably disposed in frame **34** in the same manner as the wells of the preferred embodiment, e.g. 96 wells arranged in an 8x12 array on 9.0 mm centers. However, wells **58** differ from the wells of the preferred embodiment in that wells **58** have bottom walls **60** whose upper surfaces form U-shaped well bottoms **62**. Bottom walls **60** are positioned such that the lowest point of each well bottom **62** is elevated height **E2** above bottom edge **38**.

FIGS. 8 - 9 illustrate alternative embodiments of the microplate in which the well bottoms are formed by filter material. FIG. 8 shows wells **76** having well bottoms **80** formed by individual filter plugs **78**. Wells **76** are preferably disposed in frame **34** in the same manner as the wells of the preferred embodiment, e.g. 96 wells arranged in an 8x12 array on 9.0 mm centers. Filter plugs **78** are positioned such that each well bottom **80** is elevated height **E2** above bottom edge **38**.

Each filter plug is preferably press fit between the sidewalls of a respective well. Alternatively, each filter plug may be ultrasonically bonded or adhesively bonded to the sidewalls of a respective well. One advantage of the current invention is that filter plugs **78** may have a thickness ranging up to height **E2** and still fit within frame **34**. Filter bottoms are typically thicker than non-filter bottoms, and their greater thickness often makes them difficult to fit to conventional microplates.

FIG. 9 shows wells **64** having well bottoms **68** formed by a single filter sheet **70**. Wells **64** are preferably disposed in frame **34** in the same manner as the wells of the preferred embodiment, e.g. 96 wells arranged in an 8x12 array on 9.0 mm centers. Sheet **70** is positioned such that each well bottom **68** is elevated height **E2** above bottom edge **38**. Each well **64** has respective sidewalls **66** to which sheet **70** is attached. Sheet **70** includes barrier sections **82** disposed below the sidewalls of each well. Barrier sections **82** prevent liquid transfer between adjacent wells.

Sheet **70** is preferably attached to the sidewalls of each well by ultrasonic bonding. In this process, the top surface of sheet **70** is held firmly pressed to the bottom surfaces of the sidewalls. Sheet **70** is then bonded to the sidewalls by the application of ultrasonic energy which causes the contacting surfaces to fuse together. The fusing of the contacting surfaces forms barrier sections **82**. Alternatively, sheet **70** may be press fit and adhesively bonded to frame **34**. As sheet **70** is press fit, force of contact between the top surface of sheet **70** and the bottom surfaces of the sidewalls compresses sheet **70** below the

contacting surfaces. The compression of sheet **70** forms barrier sections **82**.

FIGS. 10 - 11 illustrate a third embodiment of the invention. FIG. 10 shows a top plan view of a microplate **84** having a frame **86** and a number of well strips **88** removably disposed in frame **86**. Frame **86** has a rim **87** for holding well strips **88**. The number of well strips disposed in frame **86** at any given time may be varied to tailor microplate **84** to a specific application. Frame **86** is preferably sized to hold at least eight well strips concurrently. Each well strip **88** forms a single row of wells **90**. Wells **90** are preferably formed in each well strip such that the center of each well is spaced 9.0 mm from the center of each adjacent well. Frame **86** and well strips **88** may be easily fabricated using conventional injection molding techniques.

FIG. 11 shows a cross sectional view of one of well strips **88** disposed in frame **86**. Frame **86** includes a base **36** having a bottom edge **38**. Frame **86** extends to height **H2** above bottom edge **38**. Each well **90** formed in the well strip has sidewalls **92** and a bottom wall **94** whose upper surface forms a well bottom **96**. Each well bottom is elevated height **E2** above bottom edge **38**, as previously described in the preferred embodiment above. The advantage of the third embodiment is that it allows wells to be easily added and removed from the microplate. Other than the differences described, the operation and advantages of the third embodiment are the same as those described in the preferred embodiment above.

FIG. 12 shows a top plan view of an alternative microplate **74** having 384 wells disposed in a sixteen-by-twenty-four (16x24) rectangular array. The wells are disposed such that the center of each well is spaced a distance **Y** from the center of each horizontally and vertically adjacent well. Distance **Y** is preferably 4.5 mm. Each well in microplate **74** has an elevated well bottom which may be a flat bottom, V-shaped bottom, U-shaped bottom, or filter bottom as previously described. Microplate **74** is easily fabricated using conventional injection molding techniques. The advantage of microplate **74** is that it allows a

greater number of samples to be concurrently assayed for high throughput screening applications.

SUMMARY, RAMIFICATIONS, AND SCOPE

Although the above description includes many specificities, these should not be construed as limitations on the scope of the invention, but merely as illustrations of some of the presently preferred embodiments. Many other embodiments of the invention are possible. For example, in one alternative embodiment, the wells have tapered sidewalls to further reduce the volume of reagent required in each well.

In another embodiment, the wells are square rather than round. Round wells are illustrated in the preferred embodiment due to their predominant use in industry, but the microplate of the present invention is also effective with wells having different shapes. Further, the above embodiments describe wells arranged in 8x12 and 16x24 rectangular arrays. However, the microplate of the present invention may include any number of wells arranged in any format, e.g. 6 wells arranged in a 2x3 array, 24 wells arranged in a 4x6 array, 48 wells arranged in a 6x8 array, etc.

Therefore, the scope of the invention should be determined not by the examples given, but by the appended claims and their legal equivalents.

CLAIMS

What is claimed is:

1. A microplate for holding mixtures of reagents and samples to be assayed by emission of light signals from said mixtures to an optical detector positioned above said microplate, said microplate comprising:
   a) a frame having a bottom edge, said frame extending to a first height above said bottom edge; and
   b) a plurality of wells disposed in said frame for holding said mixtures, each said well having sidewalls and a well bottom, said well bottom being elevated a second height above said bottom edge, said second height being in a range of one half to nine tenths of said first height.

2. The microplate of claim 1, wherein said first height is in a range of 12.0 to 16.0 mm and said second height is in a range of 6.0 to 14.4 mm.

3. The microplate of claim 2, wherein said first height is about 14.35 mm.

4. The microplate of claim 1, wherein each said well has a diameter in the range of 6.0 to 8.0 mm and a depth in the range of 1.5 to 7.1 mm.

5. The microplate of claim 1, wherein each said well has a diameter in a range of 2.0 to 5.0 mm and a depth in a range of 1.5 to 4.0 mm.

6. The microplate of claim 1, wherein said wells are disposed in an eight-by-twelve rectangular array such that the center of each said well is spaced 9.0 mm from the center of each horizontally adjacent well and spaced 9.0 mm from the center of each vertically adjacent well.

7. The microplate of claim 1, wherein said wells are disposed in a sixteen-by-twenty-four rectangular array such that the center of each said well is spaced 4.5 mm from the

center of each horizontally adjacent well and spaced 4.5 mm from the center of each vertically adjacent well.

8. The microplate of claim 1, wherein said wells are formed by at least one well strip removably disposed in said frame.

9. The microplate of claim 1, wherein said well bottom is flat.

10. The microplate of claim 1, wherein said well bottom is V-shaped.

11. The microplate of claim 1, wherein said well bottom is U-shaped.

12. The microplate of claim 1, wherein said well bottom is formed by filter material.

13. The microplate of claim 12, wherein said filter material comprises an individual filter plug.

14. The microplate of claim 12, wherein said filter material comprises a filter sheet.

15. The microplate of claim 14, wherein said filter sheet includes barrier sections disposed below the sidewalls of said wells for preventing liquid transfer between adjacent wells.

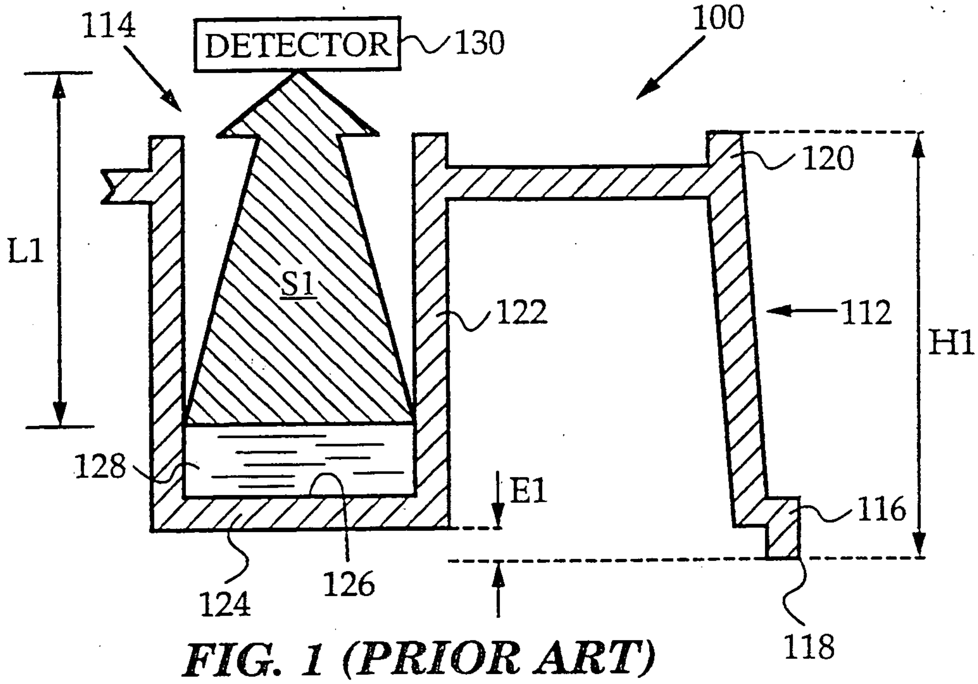

FIG. 1 (PRIOR ART)

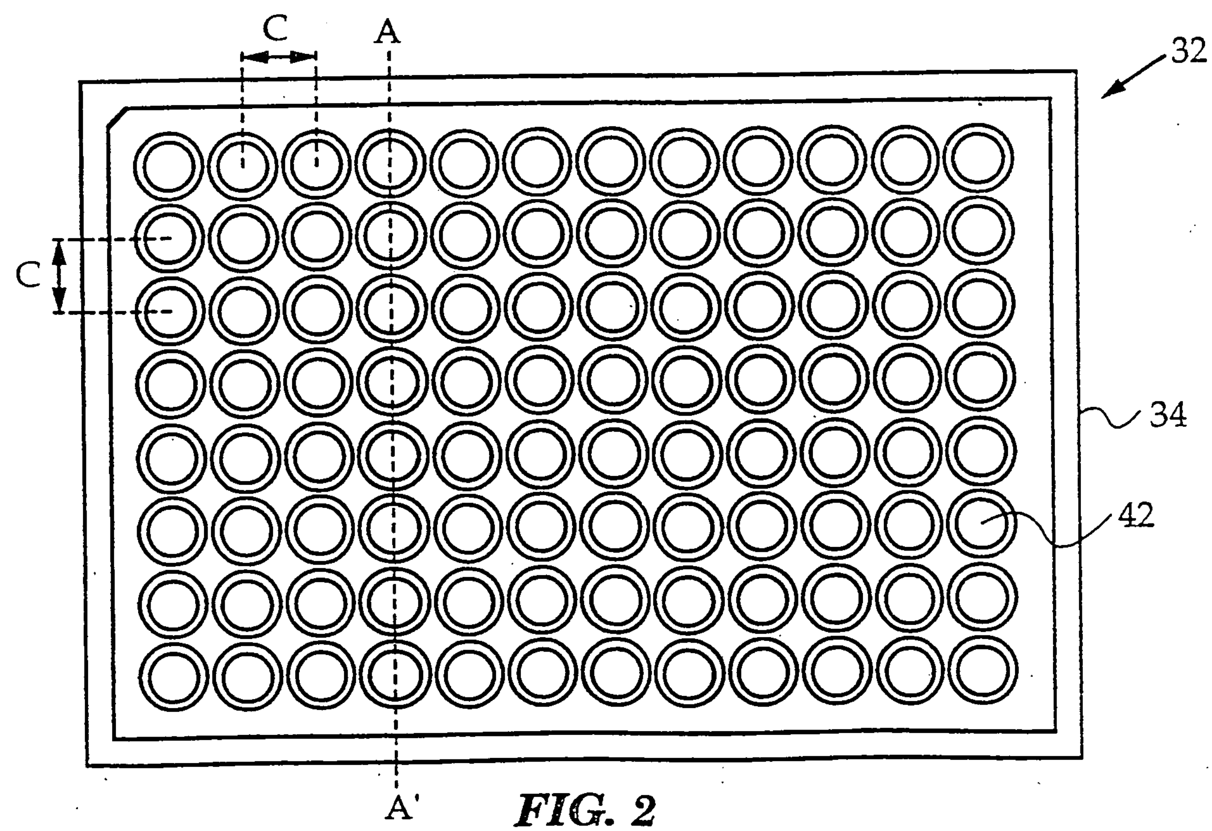

FIG. 2

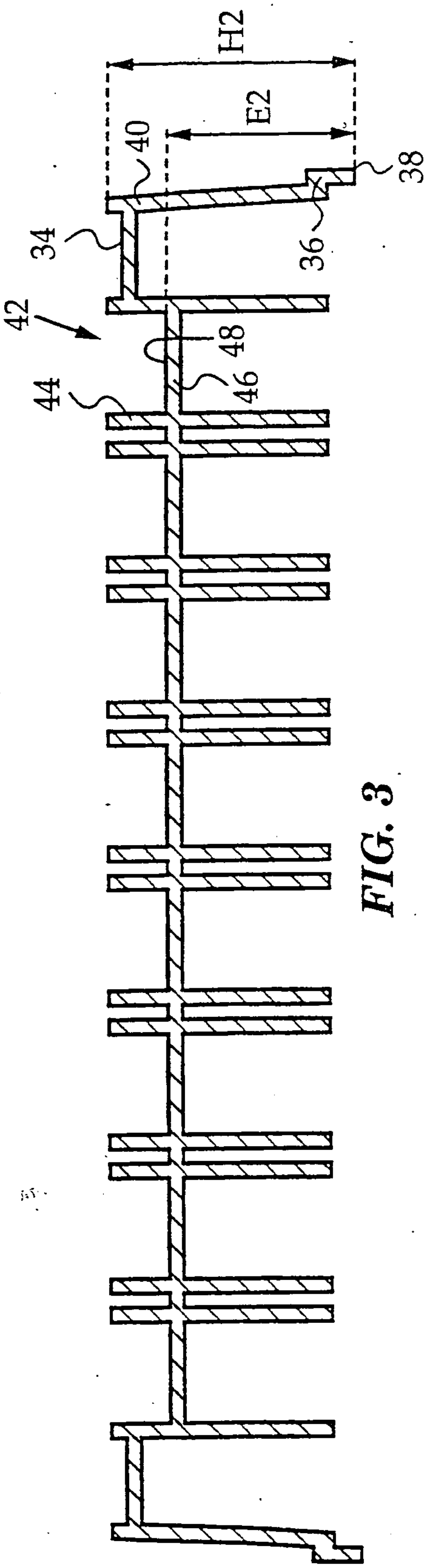

FIG. 3

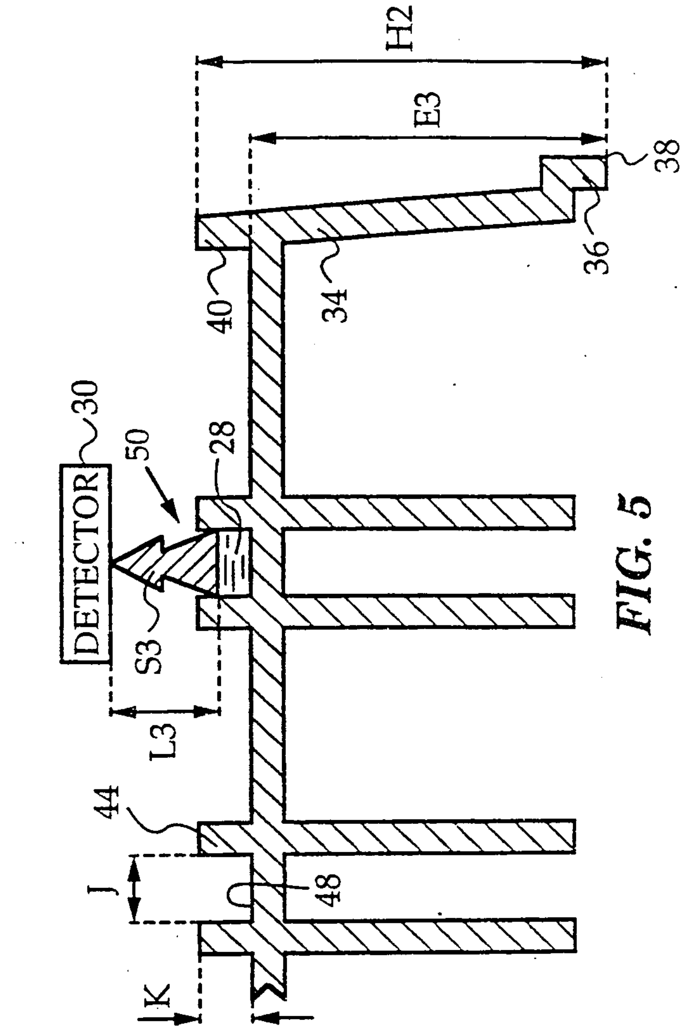

FIG. 5

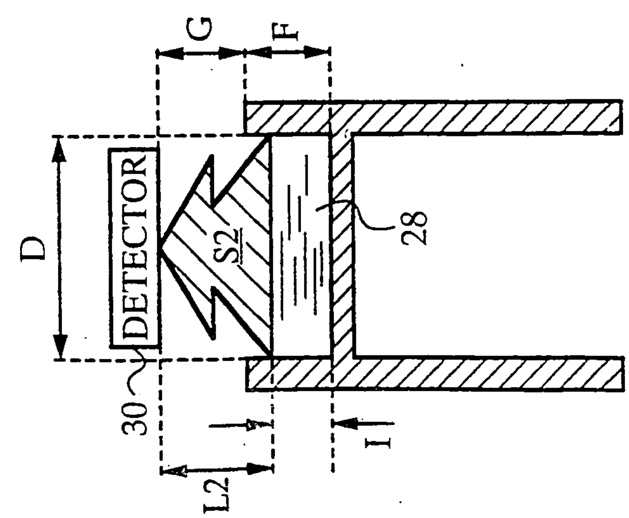

FIG. 4

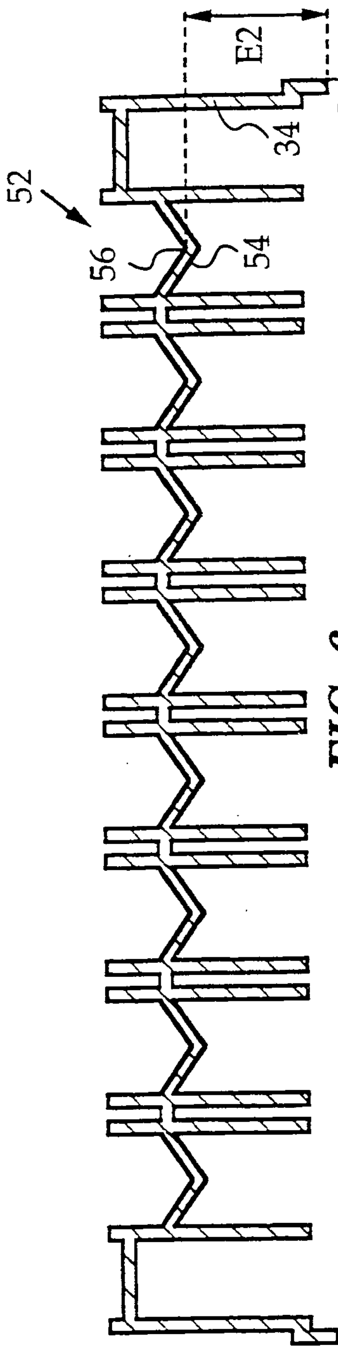

FIG. 6

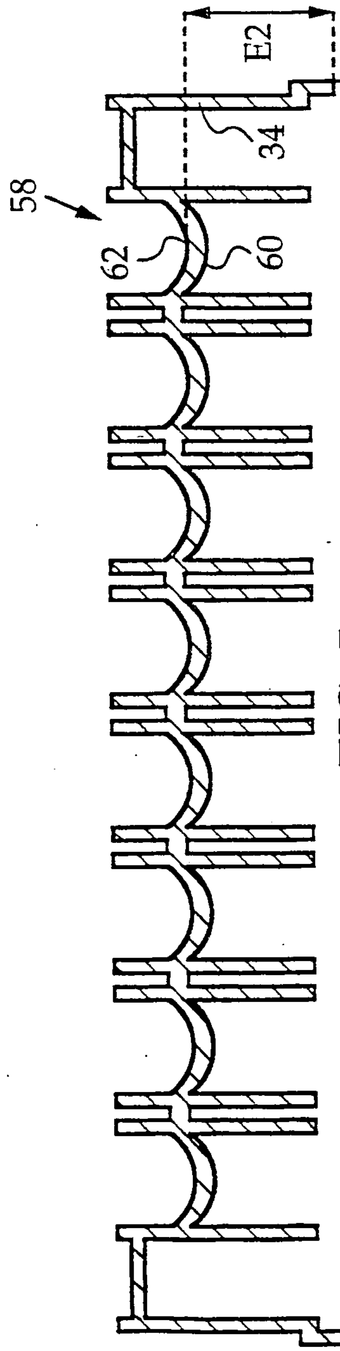

FIG. 7

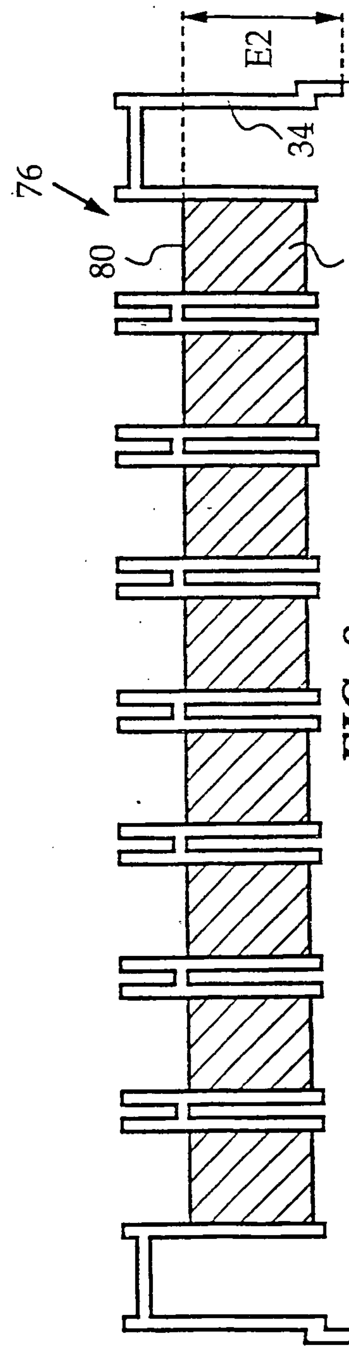

FIG. 8

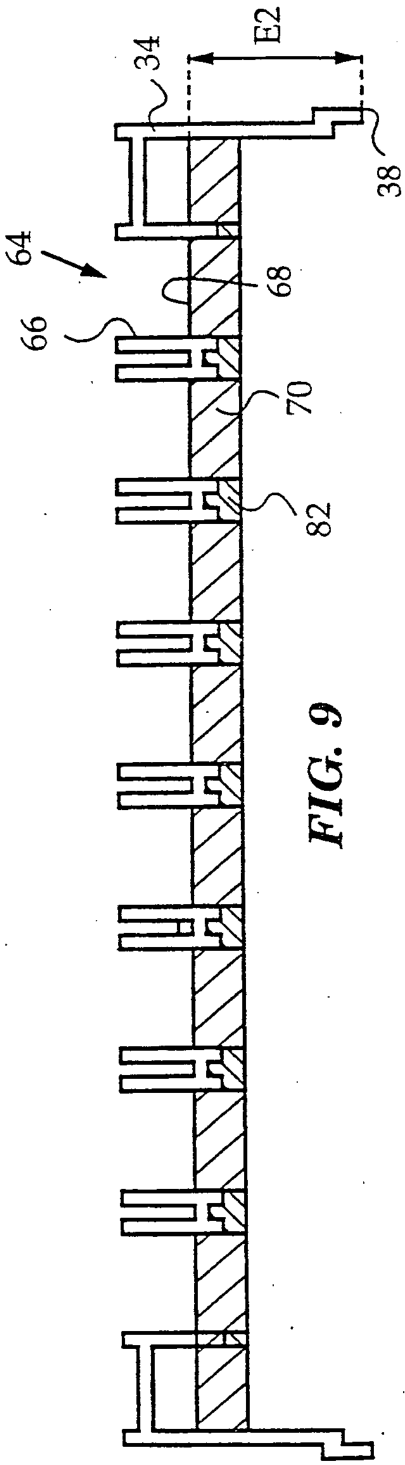

FIG. 9

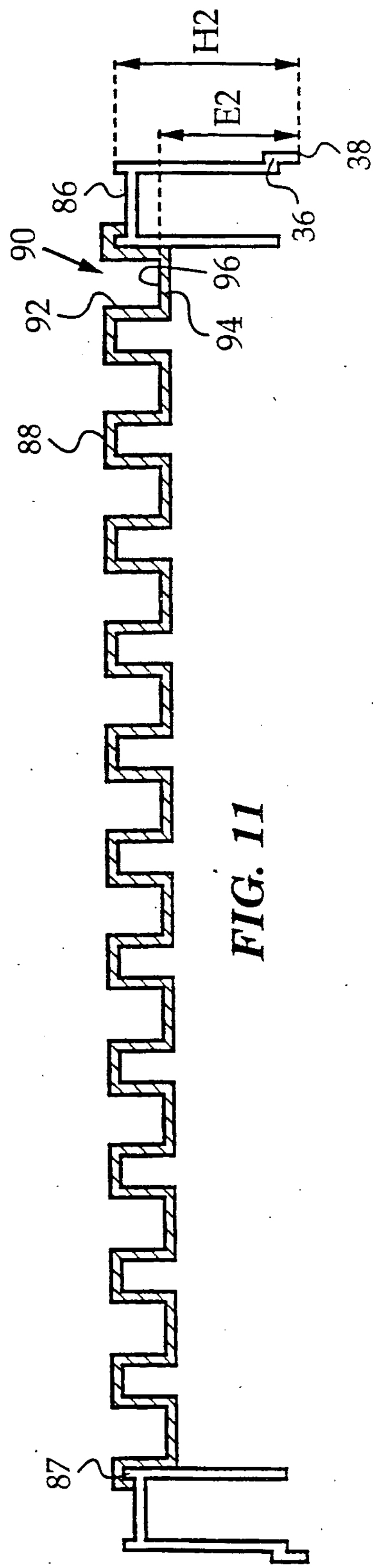

FIG. 11

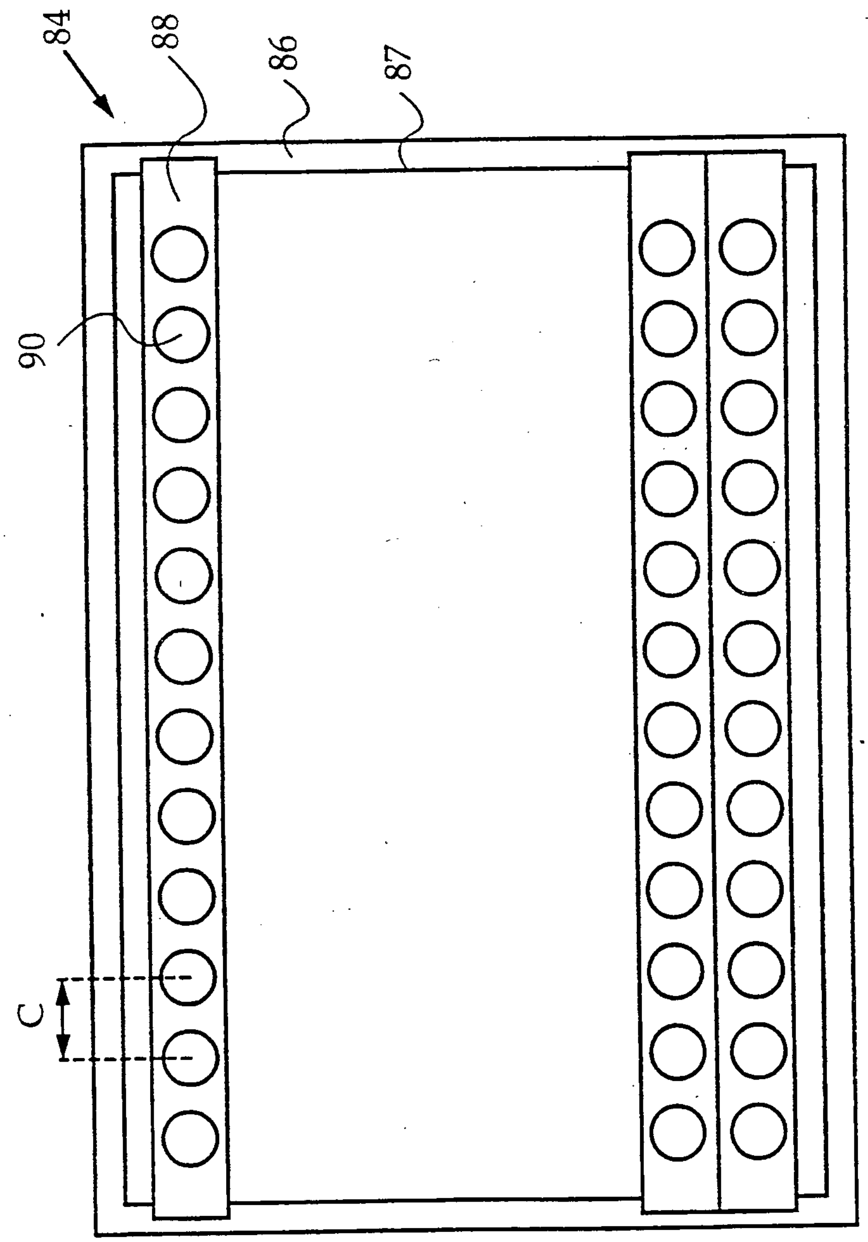

FIG. 10

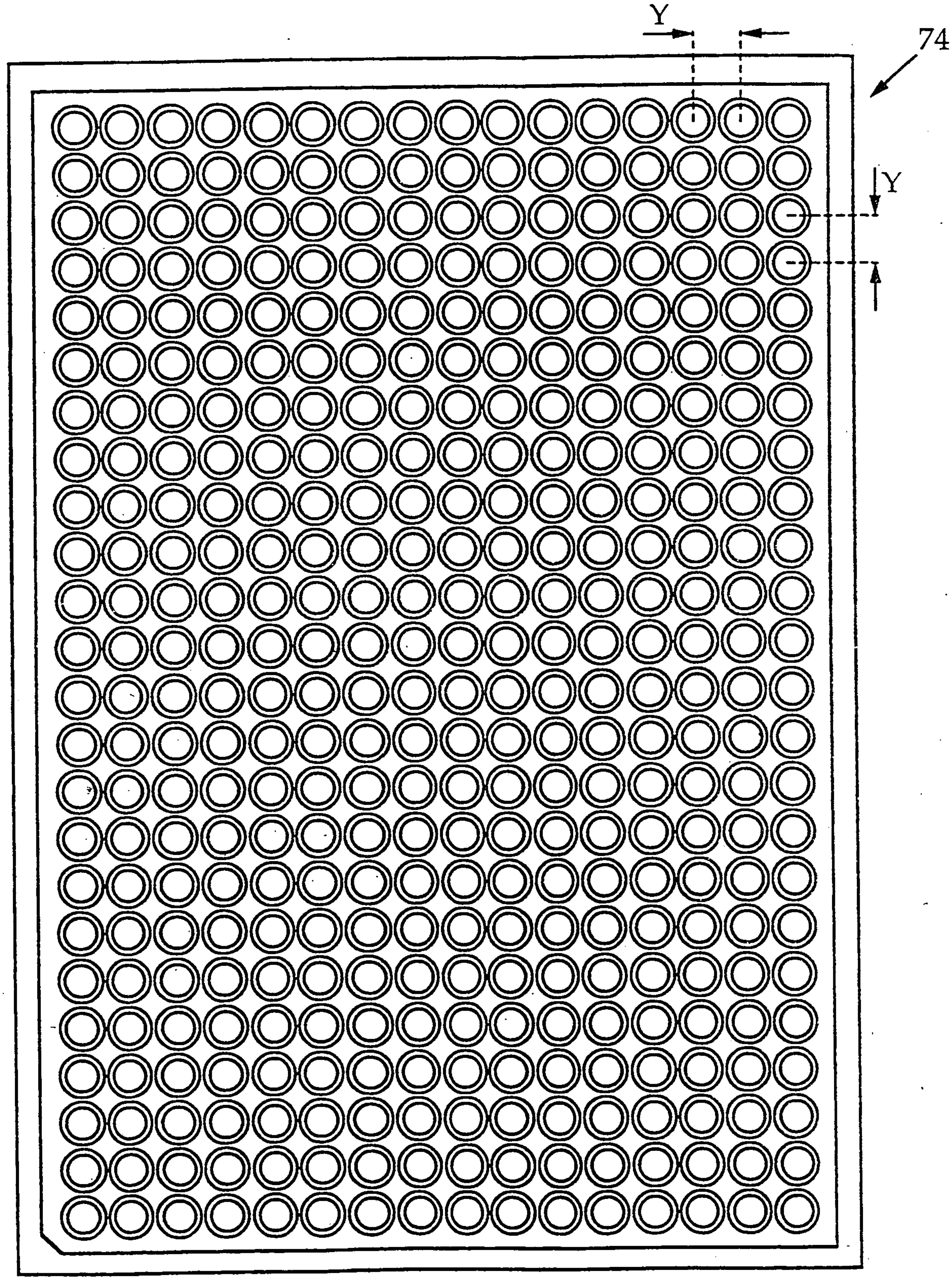

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/07505

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 21/03
US CL : 356/246
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 356/246. 436, 440

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,704,255 (JOLLEY) 3 November 1987 (03/11/87), col. 8, lines 22-32.	1-15

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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